Reviewer's report

Title: Quantitative evaluation of RASSF1A methylation in the non-lesional, regenerative and neoplastic liver.

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Reviewer: Michael C Fruehwald

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The manuscript by Gioia et al. describes the evaluation of aberrant methylation of the gene RASSF1A in tissues of different histology derived from the liver. The major (and actually pretty much only) finding of the manuscript is that aberrant methylation of RASSF1A is present in liver tissues and increases with the degree of tissue change towards malignancy and potentially also with age. The authors used methylation specific PCR (MSP), real time MSP and direct sequencing.

While the finding is important and relevant to researchers in the field of gastrointestinal disease, the paper suffers mainly from a lack in presentation.

Some points to improve are:

1) It is far from clear how many samples were run by real-time MSP and how often the experiments were repeated. The calibration curve is unfortunately not shown. It is not explained how the calibration curve has an impact on the methylation index.
2) The authors should include a detailed section on how and when the amounts of DNA were measured.
3) It is far from clear why the authors show hepatocellular carcinomas and cirrhosis in figure 1, and do not show the data on adenomas, FNH… It would be helpful if the authors could streamline the figures especially for readers not familiar with the topic of hepatic diseases.
4) The authors at times put methylation as synonymous to silencing or repression. This is not admissible as they did not test expression!
5) The regression analysis for increasing methylation with age is far from convincing. Mathematical calculations hint towards a correlation however the limited number of samples can not give proof for an age dependency. It may be helpful to study additional samples and to add tissue from children and fetuses to the study.
6) For the data presented the figures appear too numerous. I.e. why are figures 3 and 4 separate?
7) The sequencing data is not convincing at all. It has not been shown that studying the extent of methylation can be performed on genomic DNA. For good reasons most researchers rely on cloning bisulfite PCR products and then sequence a number of clones.
8) Following from number 5) the test for complete bisulfite conversion remains questionable. I would suggest sequencing some cloned samples to prove complete conversion by bisulfite. Furthermore a most sequencing analysis systems use a so called base caller, the figure may be erroneous and the data should be viewed in the raw data view and then corrected accordingly.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No

Declaration of competing interests:
'I declare that I have no competing interests'